### FSMP-09. FORMATE PROMOTES CANCER CELL INVASION AND METASTASIS VIA CALCIUM SIGNALING

Catherine Delbrouck[1,2], Vitaly I. Pozdeev[2], Anais Oudin[1], Kamil Grzyb[3,1], Laura Neises[1], Nicole Kiweler[1], Alexander Skupin[3,2], Elisabeth Letellier[2], Simone P. Niclou[1], Johannes Meiser[1]; [1]Luxembourg Institute of Health, Luxembourg, Luxembourg, [2]University of Luxembourg, Esch-Belval, Luxembourg, [3]Luxembourg Centre for Systems Biomedicine, Esch-Belval, Luxembourg

Serine catabolism via the folate cycle provides formate that is essential for nucleotide synthesis in proliferating cells. In addition to this canonical function to support biomass production in anabolic cells, we have recently demonstrated *in vitro* and *in vivo* that formate production in cancer cells is often in excess of the anabolic demand. This excess formate production is characterized by formate overflow and thus, net formate excretion into the tumor microenvironment. Interestingly, we observe increased rates of formate overflow upon different chemical perturbations that induce growth arrest. Thus, stressed cancer cells that encounter growth restriction such as upon chemotherapy, are often characterized by increased formate release rates. We demonstrated that such high formate levels in the extracellular space promote invasion of glioblastoma cells. Using *ex vivo* brain slice cultures and an orthotopic brain tumor model, we demonstrate that silencing MTHFD1L, the essential enzyme to enable formate overflow, results in decreased invasiveness of the tumor. Embarking from this observation, we investigated the underlying mechanism and now provide evidence that the formate-dependent increase of cell motility is mediated by an activation of $Ca^{2+}$ signaling. Activation of $Ca^{2+}$ signaling triggers integrin and matrix metallopeptidase (MMP) responses enabling the invasion process. Targeting either the $Ca^{2+}$ response or MMP release can suppress the formate dependent increase in invasion. Finally, we tested the effect of formate also in context of breast cancer where we were able to recapitulate our observation of increased invasiveness and, in this case, formate also promoted the metastatic potential. We conclude that excreted formate might serve as a cellular stress signal that represents a promotive trigger to support tumor escape mechanisms.

### FSMP-10. CYSTEINE INDUCES CYTOTOXICITY IN GLIOBLASTOMA THROUGH MITOCHONDRIAL HYDROGEN PEROXIDE PRODUCTION

Evan Noch[1], Laura Palma[1], Isaiah Yim[2], Bhavneet Binder[1], Elisa Benedetti[1], Jan Krumsiek[1], Olivier Elemento[1], Lewis Cantley[1]; [1]Weill Cornell Medicine, New York, NY, USA, [2]Yale University, New Haven, CT, USA

Glioblastoma (GBM) is a poorly treatable disease with high mortality. Tumor metabolism in GBM is a critical mechanism responsible for growth because of upregulation of glucose, amino acid, and fatty acid utilization. However, little is known about the specific metabolic alterations in GBM that are targetable with FDA-approved compounds. To investigate metabolic signatures unique to GBM, we interrogated the TCGA and a cancer metabolite database for alterations in glucose and amino acid signatures in GBM relative to other human cancers and relative to low-grade glioma. From these analyses, we found that GBM exhibits the highest levels of cysteine and methionine pathway gene expression of 32 human cancers and that GBM exhibits high levels of cysteine metabolites compared to low-grade gliomas. To study the role of cysteine in GBM pathogenesis, we treated patient-derived GBM cells with FDA-approved cyst(e)ine-promoting compounds in vitro, including N-acetylcysteine (NAC) and the cephalosporin antibiotic, Ceftriaxone (CTX), which induces cystine import through system Xc transporter upregulation. Cysteine-promoting compounds, including NAC and CTX, inhibit growth of GBM cells, which is exacerbated by glucose deprivation. This growth inhibition is associated with reduced mitochondrial metabolism, manifest by reduction in ATP, NADPH/NADP+ ratio, mitochondrial membrane potential, and oxygen consumption rate. Mechanistic experiments revealed that cysteine compounds induce a rapid increase in the rate of $H_2O_2$ production in isolated GBM mitochondria, an effect blocked by the $H_2O_2$ scavenger, catalase. Such findings are consistent with reductive stress, a ROS-producing process whereby excess mitochondrial reducing equivalents prevent electron transfer to oxidized electron acceptors, inducing $O_2$ reduction to $H_2O_2$. We show that cysteine-promoting compounds reduce cell growth and induce rapid mitochondrial toxicity in GBM, which may be due to reductive stress. This pathway is targetable with FDA-approved cysteine-promoting compounds and could synergize with glucose-lowering treatments, including the ketogenic diet, for GBM.

### FSMP-11. TARGETING CHOLESTEROL HOMEOSTASIS DYSREGULATION FOR THE TREATMENT OF GLIOBLASTOMA

Mingzhi Han; University of Bergen, Bergen, Norway. Shandong University, Jinan, China

Dysregulated cholesterol metabolism is a hallmark of many cancers, including glioblastoma (GBM), but its role in disease progression is not well understood. Here, we identified cholesterol 24-hydroxylase (CYP46A1), a brain-specific enzyme responsible for elimination of cholesterol through conversion of cholesterol to 24(S)-hydroxycholesterol (24OHC), as one of the most dramatically dysregulated cholesterol metabolism genes in GBM. CYP46A1 was significantly decreased in GBM samples compared to normal brain tissue. In gliomas, a reduction in CYP46A1 expression was associated with increasing tumour grade and poor prognosis. Functionally, ectopic expression of CYP46A1 suppressed cell proliferation and *in vivo* tumour growth by increasing 24OHC levels. RNA-seq revealed that treatment of GBM cells with 24OHC suppressed tumour growth through regulation of LXR and SREBP signaling. Efavirenz (EFV), an activator of CYP46A1 that is known to penetrate the blood-brain barrier (BBB), inhibited GBM growth *in vivo*. Our findings demonstrate that CYP46A1 is a critical regulator of cellular cholesterol in GBM and that the CYP46A1/24OHC axis is a potential therapeutic target.

### FSMP-12. A ROLE FOR PROLINE BIOSYNTHESIS IN HYPOXIC GLIOBLASTOMA

Lisa Vettore[1], Rebecca Westbrook[1], Jennie Roberts[1], Cristina Escribano-Gonzalez[1], Federica Cuozzo[1], David Hodson[1], Colin Watts[2], Colin Nixon[3], Daniel Tennant[1]; [1]Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, West Midlands, United Kingdom, [2]Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, West Midlands, United Kingdom, [3]CRUK Beatson Institute, Glasgow, Scotland, United Kingdom

Hypoxia is a common feature of glioblastoma, and a known driver of therapy resistance in brain tumours. Understanding the metabolic adaptations to hypoxia is key to develop new effective treatments for patients. A recent screening study highlighted Pyrroline-5-carboxylate reductase-like (PYCRL) as one of the top three genes that allowed tumour survival in hypoxia. PYCRL is one of the three enzymes involved in proline biosynthesis along with the mitochondrial pyrroline-5-carboxylate reductase 1 and 2 (PYCR1/2). The latter use glutamine as the carbon source to fuel the pyrroline-5-carboxylate (P5C)-to-proline reaction, whereas the cytosolic PYCRL is known to use ornithine to produce proline. Our investigations have shown that PYCRL differs from PYCR1 and 2 in the impact on cellular redox, which is a critical factor in hypoxic survival. Our data suggest that PYCRL activity is required for normal regulation of glioblastoma cell growth and the ability to deal with cellular stress, and that this enzyme may therefore represent a novel target in the treatment of these devastating tumours. Importantly, our study also begins to provide much-needed clarity over the network surrounding proline metabolism and redox maintenance.

### FSMP-13. HYPOXIC REGULATION OF LACTATE DEHYDROGENASE GENES (LDHA/B) IN T98 GLIOBLASTOMA MULTIFORME CELLS

Brian E. White, Russell J. Buono; Cooper Medical School Rowan University, Camden, NJ, USA

Glioblastoma multiforme (GBM) is the most common primary brain cancer and carries a poor prognosis. GBM cells exhibit extensive metabolic alterations that enhance survival and proliferation in the mixed normoxic-hypoxic tumor microenvironment. Lactate dehydrogenase (LDH) enzymes are critical mediators of the normoxic to hypoxic transition in cells. Two LDH genes (*A/B*) encode monomers that combine to form five isoenzymes (LDH1-5) with different properties for pyruvate to lactate interconversion. Hypoxic induction of *LDHA* in all cells appears to occur via HIF-1 mediated transcription. However, little is known about hypoxic regulation of *LDHB* in cancer. We report on hypoxic regulation of *LDHA/B* in T98G, a rare cell line that has both normal and neoplastic features. Human T98 GBM cell lines were cultured in a humidified incubator at 37° C and 5% $CO_2$ and were grown in normoxia (21% $O_2$) or hypoxia (95% $N_2$, 5% $C0_2$) for 72 hours. Relative expression of LDH isoforms 1-5 was assessed using native gel electrophoresis. Expression of the *LDHA* and *LDHB* genes was measured using qRT-PCR. *LDHA*-dominant isoforms (4/5) were detected in T98G cells subjected to normoxia and hypoxia via gel electrophoresis, however, *LDHB*-dominant isoforms (1/2) were not. The *LDHA/B*-equimolar isoform (3) was decreased in T98G cells subjected to hypoxia. *LDHA* gene expression was over two-fold greater than *LDHB* in normoxia ($p$ = .00256 by one-tailed Mann-Whitney U test), and over nine-fold greater in hypoxia ($p$ = .00256). *LDHA:LDHB* expression in hypoxia compared to normoxia was significantly different ($p$ = .00256). *LDHA* expression increased three-fold in hypoxia ($p$ = .00256), while *LDHB* expression decreased 0.3-fold in hypoxia ($p$ = .03288). We document *LDHB* dysregulation in T98G cells as the gene is minimally responsive to oxygen. Therapeutic strategies aimed at promoting *LDHB* expression may complement inhibition of *LDHA* and reduce GBM survival in hypoxia.